Structural Requirements for the Interdomain Linker of α Subunit of *Escherichia coli* RNA Polymerase†

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ABSTRACT: The carboxy-terminal domain of the α subunit of *Escherichia coli* RNA polymerase, which is connected with the core part of RNA polymerase through a long flexible linker, plays decisive roles in transcription activation by directly interacting with a large number of transcription factors and upstream (UP) element DNA. Here we constructed a set of mutant RNA polymerases, each containing a mutant α subunit with an altered interdomain linker. Deletion of three amino acids from the linker exhibited 50% inhibition of cAMP receptor protein- (CRP-) dependent *lac* P1 transcription. Deletion of six amino acids completely knocked out the activity. Insertion of three amino acids did not affect the activity, whereas 40−60% inhibition was observed after insertion of one, two, or four amino acids. Substitution of 10 consecutive glycine residues resulted in nearly 90% reduction of the CRP-dependent activity, whereas 50% activity was retained after substitution of 10 proline residues or a sequence expected to form a strong α-helix. Essentially the same results were obtained with UP element-dependent *rrnB* P1 transcription. These observations altogether suggest that (i) sufficient length of the interdomain linker is required for transcription activation mediated by the α carboxy-terminal domain, (ii) the linker is not totally unstructured but has structural and torsional preferences to facilitate positioning of the carboxy-terminal domain to a proper location for the interaction with CRP and UP element, and (iii) CRP-dependent activation and UP element-dependent activation share a common intermediary state in which the positioning of the α carboxy-terminal domain is of primary importance.

The α subunit of *Escherichia coli* RNA polymerase comprises two independently folded domains (*1*−*4*). The N-terminal domain (NTD;[1] residues 8−235) is required and sufficient for the assembly of core enzyme with the composition of $\alpha_2\beta\beta'$ (5−7), while the C-terminal domain (CTD; residues 249−329) plays key roles in transcription activation by interacting with either a large group of transcription factors (class I factors) such as cAMP receptor protein (CRP) and OmpR (*8*−*10*), or the upstream element (UP element), which is present in a certain class of strong promoters including ribosomal RNA promoters (*11*). The intervening sequence connecting these two domains is easily accessible to proteases (*1*, *2*) and has high motional flexibility as revealed by the measurement of NMR relaxation and the comparison of NMR signals between αCTD and intact α subunit (*12*). The flexible nature of interdomain linker is also suggested from functional studies. The αCTD is able to interact with CRP bound at widely different positions along the DNA while the RNA polymerase is bound at a fixed position of the promoter (*13*, *14*). Different surfaces of αCTD can be utilized for the interaction with upstream DNA in different initiation complexes (*15*).

Three-dimensional structures of αCTD (*3*) and αNTD (*4*) of *E. coli* RNA polymerase have been determined by NMR spectroscopy and X-ray crystallography, respectively. The αCTD has a compact globular structure consisting of four helices, two helical turns, and a long C-terminal loop with defined structure. The dimer of αNTD has an elongated flat structure. All amino acid residues that are anticipated in the interaction with two large subunits, β and β' (*6*, *7*), are exclusively located on one face of the flat structure. On the other face of αNTD dimer exist the interdomain linkers to αCTDs. Thus two αCTDs are supposed to extrude out from the core part of RNA polymerase, probably toward the upstream direction, through the long flexible linkers. The structure of *Thermus aquaticus* RNA polymerase core enzyme, which was recently solved by X-ray crystallography at 3.3 Å resolution (*16*), supports the overall topology proposed for the *E. coli* RNA polymerase. Such a molecular architecture would allow αCTDs to behave as independent motional units and interact with a variety of DNA and protein signals while the main part of RNA polymerase stays at the promoter site. Despite the possible importance of the interdomain linker in transcription activation, none of the reported structures contains the linker region. In the NMR spectroscopy of αCTD, 16 amino acid residues preceding Phe^{249} did not show any long-range NOE, suggesting that this region is either unstructured or in free motion as a whole. Likewise, both αCTD and the interdomain linker are missing in the reported structure of *T. aquaticus* core enzyme, probably due to the disorder of corresponding structures in the crystal.

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[1] Abbreviations: CRP, cAMP receptor protein; CTD, carboxy-terminal domain; NTD, amino-terminal domain; NOE, nuclear Overhauser effect; UP element, upstream element.

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To elucidate structural requirements, if any, for the interdomain linker, we analyzed in vitro functions of the reconstituted RNA polymerases each consisting of mutant α subunit with different interdomain linker. The results suggest that the interdomain linker is not totally unstructured as previously suggested but has some structural and torsional preference for the full function of αCTD in transcription activation.

EXPERIMENTAL PROCEDURES

Site-Directed Mutagenesis. All plasmids used for the expression of mutant α subunits are the derivatives of pGEMAX185, the expression plasmid for the wild-type α subunit (*8*). Plasmids pGEMAL1 (encoding Δ3 derivative of α subunit), pGEMAL4 (Δ6), pGEMAL5 (Δ9), pGEMAL6 (Δ12), pGEMAL11 (Ω1), pGEMAL12 (Ω2), pGEMAL2 (Ω3), pGEMAL13 (Ω4), and pGEMAL3 (E241A) were constructed by the method of Kunkel et al. (*17*) with deoxyuridine-containing single-stranded DNA of pGEMAX185 as the template and appropriate synthetic oligonucleotides. Plasmids pGEMAL7 (Pro), pGEMAL8 (Gly), pGEMAL9 (helix), and pGEMAL10 (amphi) were constructed by inserting synthetic double-stranded DNA into the unique *Afl*II site that had been created on the plasmid pGEMAL6 (see Figure 1). Plasmids pGEMAL7R (rev1), pGEMAL8R (rev2) and pGEMAL9R (rev3) were the byproducts of pGEMAL7, pGEMAL8, and pGEMAL9, respectively, having the same insert DNA in the opposite direction. All constructions were confirmed by DNA sequencing of the plasmids and matrix-assisted laser desorption−ionization/time-of-flight mass spectrometry of the purified proteins.

Protein Expression and Purification. Wild-type α, *β*, *β′*, and σ^{70} subunits were expressed and purified by the established methods (*8*, *18*). All α derivatives were expressed in *E. coli* strain BL21 (λDE3) and purified by essentially the same method as wild-type α subunit except that a Poros HQ/H column (PerSeptive Biosystems) was used instead of a Protein Pak G-DEAE column for HPLC purification. CRP was purified from *E. coli* cells harboring pHA7 plasmid (*19*) by the method of Eilen et al. (*20*).

Reconstitution of Core Enzyme and Holoenzyme. Wild-type and mutant core enzymes were reconstituted in vitro and separated from unassembled subunits and subassemblies as described (*18*). Holoenzymes were prepared by incubating reconstituted core enzymes with a 4-fold molar excess of σ^{70} for 20 min at 30 °C.

Transcription in Vitro. In vitro transcription from *lac* promoter with and without the addition of CRP was carried out on a linear template under the conditions described previously (*8*). Another template containing CRP-independent *lacL8UV5* promoter was added to the reaction as internal reference. Transcription from *rrnB* P1 promoter with and without the UP element was carried out on closed circular plasmid templates in which the promoter sequence is followed by the strong *rrnB* T1T2 terminators. Open complex formation and subsequent elongation reaction with *rrnB* P1 promoter was carried out at 25 °C instead of 37 °C. Other conditions were the same as in the case of *lac* transcription. Transcripts were separated on a denaturing polyacrylamide gel and analyzed with a Bioimage Analyzer BAS2000 (Fujix). DNA templates used were a 205 bp *Pvu*II−*Xba*I fragment of pUC19 carrying the wild-type *lac* promoter (*8*); a 207 bp *Eco*RI fragment of pKB252 carrying the *lacL8UV5* promoter (*21*); the plasmid pWR52 carrying the *rrnB* P1 promoter sequence from −88 to +1 with 1 bp deletion at position −72 (*22*); and the plasmid pWR55 carrying the *rrnB* P1 promoter sequence from −41 to +1 with a vector sequence substituting for the UP element (*11*).

RESULTS

Construction of Linker Mutations. Sixteen different species of α-subunit derivatives each having deletion, insertion, or amino acid substitutions in the interdomain linker were constructed. Figure 1 summarizes the nucleotide as well as the amino acid sequence of the derivatives. The first eight derivatives have various lengths of deletion or insertion at the middle of the linker. Δ3, Δ6, Δ9, and Δ12 derivatives have 3, 6, 9, and 12 amino acid deletions, while Ω1, Ω2, Ω3, and Ω4 derivatives have 1, 2, 3, and 4 amino acid insertions, respectively. Pro and Gly derivatives have 10 consecutive proline and glycine residues, respectively, which substitute for almost the entire region of the interdomain linker between Asp^{236} and Glu^{245} without changing the total number of amino acids. Poly-L-proline sequence is known to form a linear type II helix (*23*, *24*) while polyglycine sequence is expected to prevent the formation of any particular secondary structure in the linker region. In helix and amphi derivatives, the amino acid sequence of the linker region was designed so as to form a strong α-helix. Figure 2A shows the plot of helix probability at each amino acid position calculated by the statistical method of Garnier et al. (*25*). The wild-type sequence was predicted to have two short helical segments at the N-terminal and central part of the linker, but the formation of long helical structure is prevented by the presence of two proline residues at positions 240 and 247. As the result of replacement of these proline residues, as well as several other amino acid substitutions, both helix and amphi derivatives acquired high helix probability throughout the linker region. In addition to the high probability of α-helix formation, the amphi derivative was designed to have an amphiphilic nature, having charged amino acid residues on one side of the helix and hydrophobic amino acid residues on the other side (Figure 2B). Three other derivatives, rev1, rev2, and rev3, which have unrelated sequences in the linker region (see Experimental Procedures), and the E241A derivative, which has a single amino acid substitution at position 241, were also used for comparison.

These α derivatives, together with the wild-type α subunit, were used to reconstitute respective core enzymes. All α derivatives showed essentially the same efficiency of core enzyme reconstitution as the wild-type α subunit (data not shown), suggesting that none of the modifications in the interdomain linker interfere with the assembly function of αNTD. When σ^{70} holoenzymes were reconstituted and assayed on the factor-independent *lacL8UV5* promoter, all mutant holoenzymes except the amphi derivative showed the same level of transcription activity as the wild-type enzyme (see Figure 3). The amphi holoenzyme always showed slightly lower activity (about 75% of the wild-type enzyme) in six independent measurements.

Effect of Linker Length on CRP-Dependent lac P1 Transcription. CRP-dependent transcription activity of the wild-type and mutant RNA polymerases was determined with

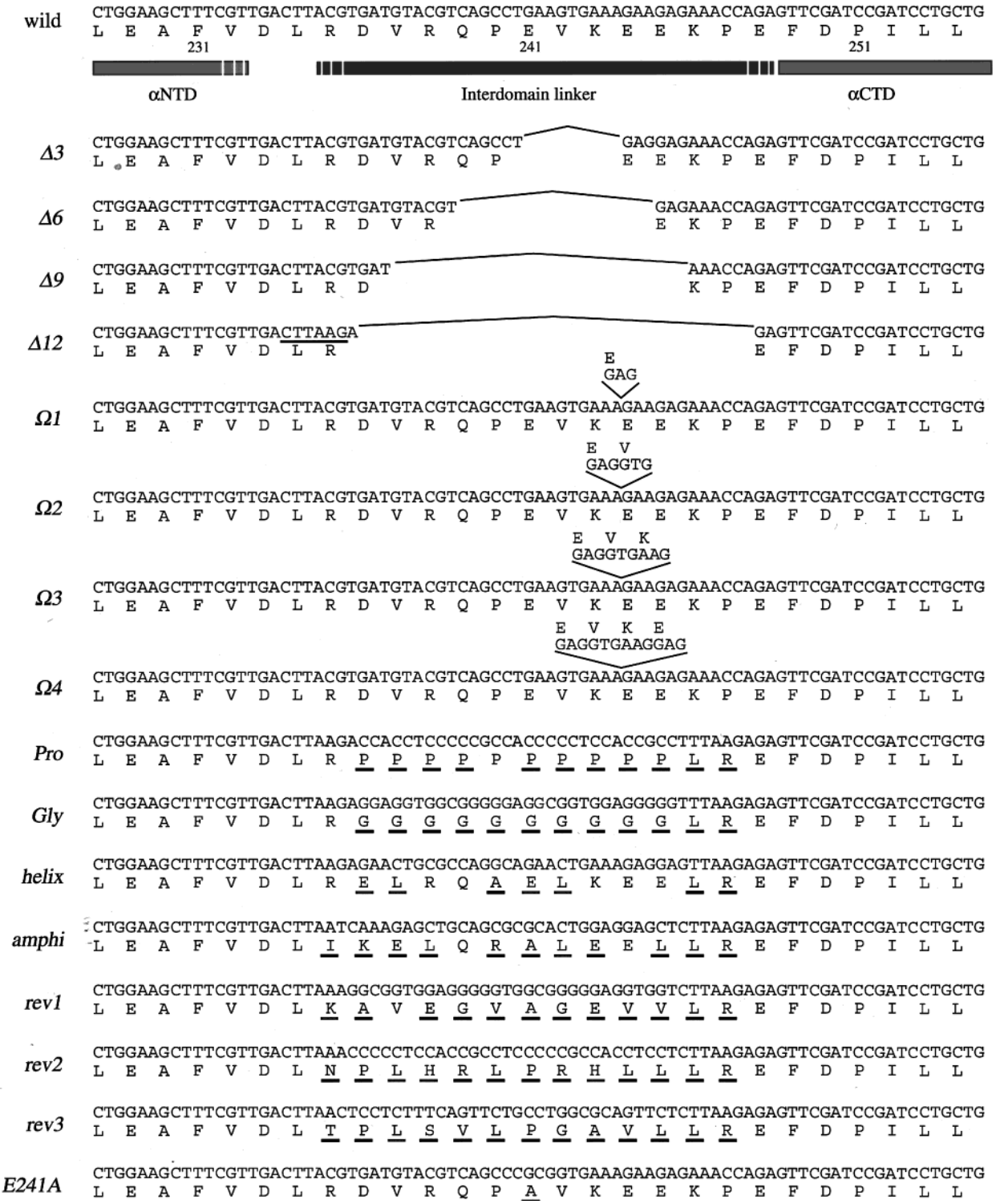

FIGURE 1: Nucleotide and amino acid sequences of mutant α subunits. The name of the protein assigned for each mutant derivative is italicized on the left. Amino acid numbering is based on the wild-type sequence, which is shown at the top. The extent of interdomain linker estimated by NMR analysis (*12*) was shown below the wild-type sequence. Conservative and nonconservative amino acid substitutions are shown by gray and black underlines, respectively. A unique *Afl*II site introduced in the pGEMAL6 plasmid encoding Δ12 derivative is also underlined (see Experimental Procedures).

a linear DNA template that contains overlapping *lac* P1 and *lac* P2 promoters. A small amount of *lacL8UV5* template was added as an internal reference. Figure 3A shows the gel electrophoresis pattern of the transcripts produced by holoenzymes with deletion or insertion in the interdomain linker. The α-235 derivative (*8*), which lacks the entire region of αCTD and the interdomain linker, was used for comparison. The level of *lac* P1 transcription was corrected for experimental fluctuation using the level of *lacL8UV5* transcription and plotted in Figure 3B as relative value, taking the activity of wild-type reconstituted enzyme in the presence of CRP as 100%.

Deletion of three amino acids at the middle of the interdomain linker (Δ3) exhibited more than 50% reduction of the CRP-dependent *lac* P1 transcription. The CRP-dependent activity was completely lost when six or more

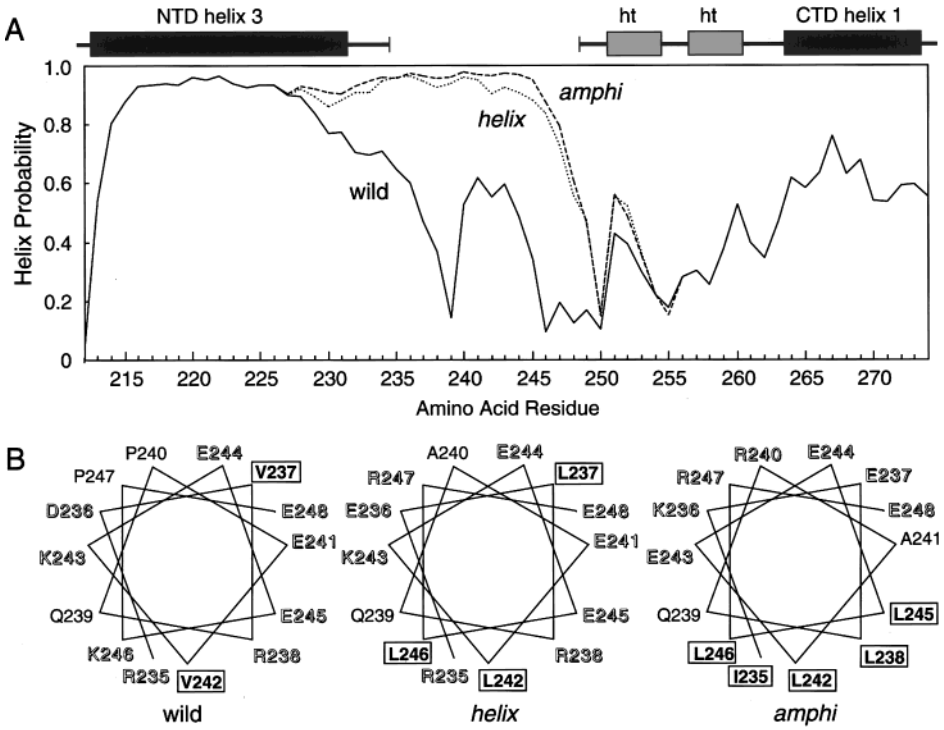

FIGURE 2: Expected secondary structure of interdomain linker of wild-type and two mutant α subunits. (A) Helix probability at each amino acid position from 212 to 274 was calculated for the wild-type α subunit, the helix derivative, and the amphi derivative. The statistical method of Garnier et al. (*25*) was used for the calculation with a window size of 17. Positions of α-helices and short helical turns (ht) identified by either X-ray crystallography (*4*) or NMR spectroscopy (*3*) are indicated at the top. (B) Helical wheel projection from amino acid position 235 to 248 is shown for the wild-type α subunit (left), the helix derivative (middle), and the amphi derivative (right). Charged amino acid residues are shown with open letters, and hydrophobic amino acid residues are boxed.

amino acids were deleted (Δ6, Δ9, and Δ12). These results indicate that sufficient length of the interdomain linker is required for the αCTD to function in transcription activation. On the other hand, only a small effect, if any, was observed when three amino acids were inserted at the middle of the interdomain linker (Ω3). In contrast, 43%, 61%, and 60% reduction of the CRP-dependent activity was observed when one (Ω1), two (Ω2), and four (Ω4) amino acids were inserted, respectively. This apparent periodicity may indicate that the length of the interdomain linker, or the distance between αNTD and αCTD, is not the sole determinant to support the function of αCTD, but instead the positioning of αCTD to proper orientation is important for the interaction with CRP. It also suggests that the linker region is not totally free for rotation along the axis of polypeptide chain.

Effect of Amino Acid Substitutions on CRP-Dependent lac P1 Transcription. The same type of experiments were carried out for RNA polymerases with amino acid substitutions in the interdomain linker of α subunit. The Pro derivative, which is expected to form type II helix structure in the linker region, retained 50% activity of the CRP-dependent *lac* P1 transcription (Figure 3C,D), suggesting that a rigid structure like the poly-L-proline helix can substitute, at least in part, for the natural linker sequence. In sharp contrast, the Gly derivative, supposedly with no particular secondary structure, showed less than 15% activity compared with the wild-type enzyme. The helix derivative, designed to form α-helix structure in the linker region, retained nearly 50% activity like the Pro derivative. All these results strongly suggest that an ordered structure like polyproline helix or α-helix in the interdomain linker is not inhibitory to, or even necessary for, the function of αCTD. The most severe impairment of CRP-dependent *lac* P1 transcription, comparable to that with the complete deletion of αCTD (α-235), was observed with the amphi derivative. The amphi derivative also showed lower specific activity on the factor-independent *lacL8UV5* template (mentioned above) as in the case of the α-235 deletion (*8*). The highly hydrophobic surfaces of the amphiphilic linkers (Figure 2B) may interact with each other or with some other part of RNA polymerase, resulting in the complete blockage of the function of αCTD. All three derivatives, rev1, rev2, and rev3, which have unrelated sequences in the linker region, showed markedly reduced activity in the CRP-dependent *lac* P1 transcription, confirming the importance of sequence context of the linker. The E241A derivative with a single amino acid substitution did not show any noticeable effect.

Transcription from *lac* P2 promoter in the absence of CRP was also affected to various extent by deletions, insertions, and amino acid substitutions in the interdomain linker (Figure 3A,C). Effect of each mutation on the *lac* P2 transcription was similar to that on *lac* P1 transcription in the presence of CRP, although the effect of longer deletions was much less pronounced in the case of *lac* P2. Parallel response of the CRP-dependent *lac* P1 transcription and the CRP-independent *lac* P2 transcription to various mutations in the α subunit was also observed previously (*8*, *26*). Striking similarity between the *lac* P2 promoter and the *rrnB* P1 promoter on linear template (see below) in the response to various linker mutations further supports the previous assumption that the *lac* P2 promoter possesses a functional UP element (*27*, *28*).

Effect of Linker Mutations on UP Element-Dependent rrnB P1 Transcription. The effect of mutations in the interdomain

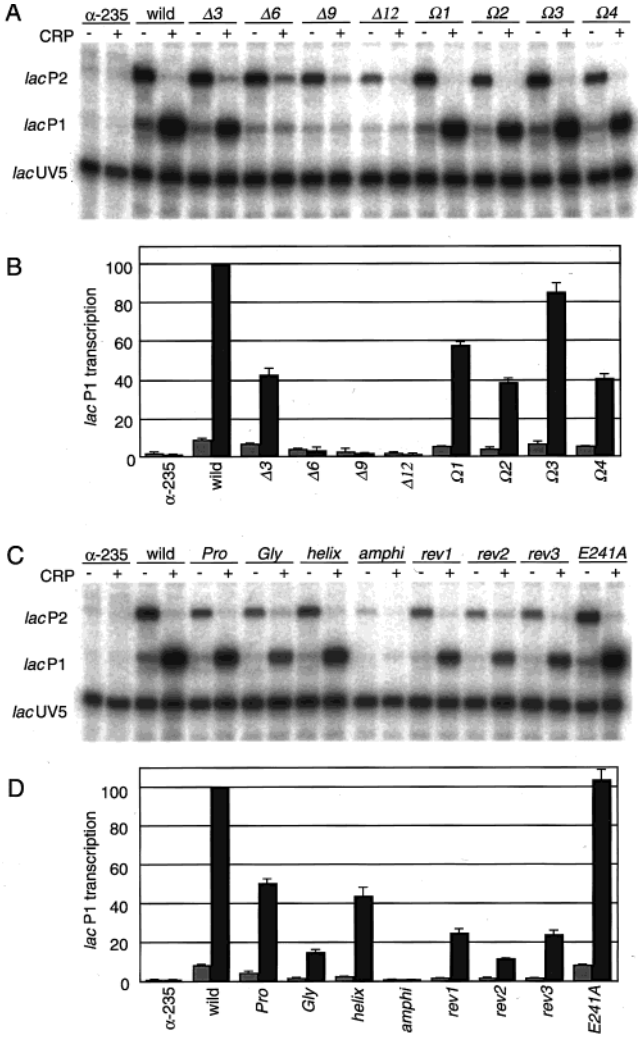

FIGURE 3: Effect of linker mutations on CRP-dependent *lac* P1 transcription. (A, C) Mixture of wild-type *lac* template (0.2 pmol), mutant *lacL8UV5* template (0.05 pmol), cAMP (10 μM), and CRP (2.5 pmol of dimer) where indicated was incubated at 37 °C for 10 min in a total volume of 30 μL. Reconstituted RNA polymerase (1 pmol in 5 μL) containing wild-type or mutant α subunit was added and the incubation was continued for 25 min for the formation of open complexes. Substrate mixture (15 μL) with heparin was then added and the incubation was continued for another 5 min for single-round RNA synthesis. RNA products were separated by electrophoresis on 8% polyacrylamide gel containing 8 M urea. The name of the α subunit used for the reconstitution of RNA polymerase is shown at the top. Positions of specific transcripts are shown on the left. (B, D) The amount of *lac* P1 transcript was corrected for experimental fluctuation using the amount of *lacL8UV5* transcript in the same reaction and plotted as relative value, taking the activity of wild-type enzyme in the presence of CRP as 100%. Gray and black bars show the activities in the absence and the presence of CRP, respectively. The average of 3−4 independent experiments is shown. Error bars represent standard deviations.

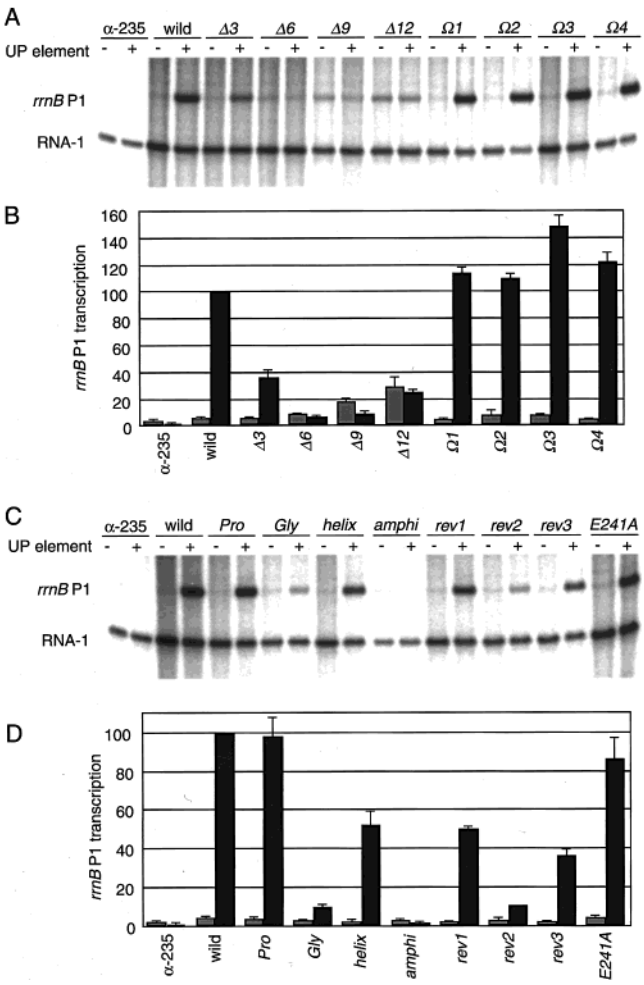

FIGURE 4: Effect of linker mutations on UP element-dependent *rrnB* P1 transcription. (A, C) Mixture of plasmid template (0.2 pmol) and reconstituted RNA polymerase (1 pmol) was incubated at 25 °C for 30 min in a volume of 35 μL. Supercoiled plasmids containing *rrnB* P1 promoter sequences with and without the UP element (pWR52 and pWR55, respectively) were used as templates. After addition of substrate−heparin mixture (15 μL), the incubation at 25 °C was continued for another 5 min. RNA products were separated by electrophoresis on 6% polyacrylamide gel containing 8 M urea. The same set of reconstituted RNA polymerases were used as in Figure 3. The positions of *rrnB* P1 and RNA-1 transcripts were shown on the left. (B, D) The amount of *rrnB* P1 transcript was plotted as relative value, taking the activity of wild-type enzyme on pWR52 template as 100%. Gray and black bars show the activities in the absence (pWR55) and the presence (pWR52) of functional UP element, respectively. The average of 3 independent experiments is shown. Error bars represent standard deviations.

linker of the α subunit on UP element-dependent *rrnB* P1 transcription was tested in vitro. Closed circular plasmids were used as templates because the *rrnB* P1 promoter on linear DNA can form a stable open complex only in the presence of initiating nucleotides ATP and CTP (*29*), and the complex formed in the presence of ATP and CTP may not be a natural intermediate of *rrnB* P1 transcription initiation (*30*). As can be seen in Figure 4, the overall responses of the UP element-dependent *rrnB* P1 transcription to various linker mutations were very similar to those of the CRP-dependent *lac* P1 transcription (Figure 3), including the severe defect in activated transcription for Δ6, Δ9, and Δ12 deletions, the higher transcription activity of the Ω3 derivative compared with other insertion derivatives (Ω1, Ω2, and Ω4), and the substantial and complete loss of activated transcription in the Gly and amphi derivatives, respectively.

For direct comparison with the CRP-dependent *lac* P1 transcription (Figure 3), which was conducted on linear template, the transcription assay was also carried out on linear DNA templates containing the *rrnB* P1 promoter with and without the UP element (data not shown). ATP and CTP were included in the step of open complex formation. The effect of each mutation was qualitatively similar to that observed with closed circular templates (Figure 4) except that the *rrnB* P1 transcription on linear DNA is far more tolerant to longer deletions of interdomain linker than the

transcription on closed circular DNA. The level of *rrnB* P1 transcription on linear template with UP element was 86%, 50%, 25%, and 10% that of the wild-type enzyme for Δ3, Δ6, Δ9, and Δ12 derivatives, respectively.

The plasmids used as templates direct the synthesis of RNA-1 transcript, which was initially intended to be used as an internal reference. However, the transcription of RNA-1 per se was found to be affected by the linker mutations to various extent (Figure 4A,C), in sharp contrast to the *lacL8UV5* promoter, whose transcription was affected only by the amphi mutation. For this reason, no correction for experimental fluctuation was made in the data presented in Figure 4B,D. Interestingly, the RNA-1 transcription was not affected by Δ6, Δ9, and Δ12 deletions, which caused severe defects in the CRP-dependent *lac* P1 transcription and the UP element-dependent *rrnB* P1 transcription, implying that the mechanism underlying the reduced transcription of RNA-1 is different from that in other cases.

DISCUSSION

All data presented in this paper are consistent with the idea that the interdomain linker of the α subunit is not totally unstructured as initially considered (*1*, *31*) but has some structural and torsional preferences for αCTD to express the full activity in transcription activation. The 3 amino acid periodicity observed in the influence of amino acid insertions (Ω1, Ω2, Ω3, and Ω4) roughly corresponds to the 3.6 amino acid periodicity of typical α-helix, suggesting that the functional conformation of the interdomain linker contains a helixlike structure(s). Calculation of the helix probability by the method of Garnier et al. (*25*) predicted the presence of short helical segments at N-terminal (232−237) and central (240−244) parts of the linker (Figure 2). Other prediction methods of the protein secondary structure based on multiple alignment of similar sequences, including the standard PHD method of Rost and Sander (*32*), did not produce convincing results because of the lack of apparent similarity of linker sequences among distantly related bacteria and chloroplasts. In the NMR spectroscopy of the C-terminal fragment of α, some sequential and medium-range $^1H-^1H$ NOEs reminiscent of ordered structures were observed[2] in the region between Asp^{233} and Glu^{248}, although no particular secondary structure could be assigned (*3*). These results, together with the observed motional flexibility (*12*), may suggest that the interdomain linker is in a dynamic equilibrium among different conformations each containing an ordered structure(s) like α-helix, thus facilitating the positioning of αCTDs to appropriate sites and orientations in various initiation complexes. Alternatively, an ordered structure in the interdomain linker may be induced only after the completion of initiation complex formation, and such structure is important for some postrecruitment events in the activation process of transcription initiation.

The higher activity of Ω3 derivative than those of the other insertion derivatives might be ascribed to the difference of charge distribution in the linker region, since the Ω3 derivative has the same net charge (−1) as the wild-type α in the region between Arg^{235} and Glu^{248}, while other insertion derivatives are more negatively charged in this region. This possibility is, however, unlikely because the E241A derivative, in which a glutamic acid at the center of the interdomain linker is replaced with alanine, was as active as the wild-type enzyme in all transcription experiments. Pro, Gly, helix, amphi, and all rev derivatives have a common two amino acid substitution (Lys-Pro to Leu-Arg) at the C-terminal end of the linker (positions 246 and 247) due to partial duplication of the *Afl*II site. The possibility cannot be excluded that the mutation has some additional influence on the enzyme activity, especially in the case of Pro and Gly derivatives.

The αCTD is involved in transcription activation by both transcription factors and DNA UP element. Random and site-directed mutagenesis studies (*26*, *33*, *34*), together with chemical shift perturbation experiments (*3*), revealed that the amino acid residues responsible for the CRP-dependent activation of *lac* P1 transcription and the amino acid residues involved in the interaction with *rrnB* P1 UP element are very close to, or overlapping with, each other on the same surface of αCTD. In addition, we showed in this study that any change in the length or the flexibility of interdomain linker of α subunit produces almost equivalent effects on these two activation systems. These observations altogether suggest that the same structural elements on αCTD can interact with either the protein factor (CRP) or the DNA element (UP element) depending on the architecture of initiation complex, or these two activation systems share a common intermediary state in the activation pathway. Studies by DNA footprinting (*13*, *35*) and iron-mediated DNA cleavage (*14*) indicated that at least one of the αCTDs is in close contact with DNA at a region just upstream of the −35 hexamer in many CRP-activated initiation complexes. Our recent study by fluorescent monitoring of the conformation and/or the environment of αCTD in various transcription complexes (*15*) also suggested the importance of the interaction between αCTD and upstream DNA in CRP-dependent transcription activation and the primary involvement of helix 1 on αCTD in this interaction as in the case of αCTD−UP element interaction. Such interaction of αCTD with upstream DNA might be the critical step in transcription activation common to UP element-mediated activation and at least part of factor-mediated activation.

Despite the general similarity between the CRP-dependent transcription (Figure 3) and the UP element-dependent transcription (Figure 4) in the response to various linker mutations, several differences can be pointed out. One obvious difference was found in the relative activity of insertion derivatives compared with the wild-type enzyme. All four insertion derivatives exhibited higher activity than the wild-type enzyme in the UP element-dependent *rrnB* P1 transcription (Figure 4), whereas none of the insertion derivatives exceeded the wild-type level in the case of CRP-dependent *lac* P1 transcription (Figure 3). It seems that the effective length of the natural interdomain linker is not necessarily optimized for the UP element-dependent transcription of *rrnB* P1 promoter on supercoiled template. Difference of the optimal linker length in different transcription systems was recently reported by Meng et al. (*36*). Also noticeable are the differences in the activity of Pro and Gly derivatives. The Pro derivative was as active as the wild-type enzyme in the UP element-dependent *rrnB* P1 transcription (Figure 4), whereas it showed 50% reduction in the CRP-dependent *lac* P1 transcription (Figure 3). Inversely, the Gly

[2] Y. H. Jeon et al., unpublished observation.

substitution caused a more severe effect on the *rrnB* P1 transcription than on the *lac* P1 transcription. The UP element-dependent transcription, but not the CRP-dependent transcription, appears to favor a more rigid structure like the polyproline helix in the linker region. Alternatively, the higher activity of the Pro derivative in the *rrnB* P1 transcription might be attributed to the longer reach of the expected polyproline helix (*23*) than the natural linker sequence, which compensates the intrinsic partial defect of the Pro derivative in transcription activation.

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